UNIVERSITI PUTRA MALAYSIA

NUTRITIVE VALUE OF FERMENTED AND EXTRUDED CANOLA MEAL AS A DIETARY INGREDIENT FOR BROILER CHICKENS UNDER UNHEATED AND HEATED CONDITIONS

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By

AHMED ABED WADI ALJUOBORI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November 2014

DEDICATION

With appreciation and respect, this thesis is dedicated

To my parents, brother and sisters who supported and inspired me with confidence and ambitions

To my beloved wife and kids, who supported me through the valley of darkness with light of hope and the sacrifices

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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November 2014

Chairman: Professor Zulkifli B Idrus, PhD

Faculty : Agriculture

The amino acid profile of canola meal (CM) protein compares favorably with that of soybean meal protein. However, it contains antinutritional factors, and high dietary fibre which may limit its utilisation in poultry feeding. Thus, there is a need to investigate possible methods to improve the nutritional value of CM. There is also a concern of feeding CM to poultry under hot climate because of its high fibre content. The present work determined the effects of extrusion and fermentation on the content of various nutrients and anti-nutrients in CM. The response of broiler chickens to various levels of untreated, extruded (EM) and fermented (FCM) CM under unheated and heated conditions were elucidated. The first study was to determine the effect of extrusion process on apparent metabolizable energy (AME), crude protein (CP) and amino acid (AA) digestibility of CM in broiler chickens. The results showed that ECM had greater AME (2595 vs 2243 kcal/kg) compared to CM. Extrusion also significantly enhanced apparent ileal digestibility of CP and some of AA such as Asp, Glu, Ser, Thr and Trp. The second digestibility study was to investigate the potential of Lactobacillus salivarius solid state fermentation for reduction of glucosinolate content in canola meal (CM) as well as the improvement of its nutrient digestibility for broiler chickens. Nutrients ileal digestibility was tested using 42-day-old broilers fed either CM or FCM as the sole source of energy and protein. The digestibility coefficient was improved significantly for crude protein, Met, Cys, Arg, Asp, Glu, and Ser in FCM compared to CM. However, apparent metabolisable energy of CM was not affected by fermentation. Three feeding trials were conducted to determine the effect of various levels of dietary inclusion of untreated CM, ECM and FCM on performance, carcass yield, body temperature and plasma levels triiodothyronine (T_3) and tetraiodothyronine (T_4) in broiler chickens under chronic heat stress. Treated CM was included in the starter and finisher diets at 0, 10, 20, and 30% from 1 to 35 days of age. All diets were isocaloric and isonitrogenous. From 29 to 35 day an equal number of birds from each dietary group were exposed to either 23±1^{o}C (unheated) or 36±1°C (heated) throughout. Inclusion of untreated of CM at 10% or above retarded weight gain and FCR of broiler chicks during the starter phase. CM can be added up to 30% in the finisher diet of heated chickens without any adverse effect on growth performance but not under unheated condition. Carcass parameters, T_3 and T_4 were not affected by diet. Heated birds had lower carcass yield and T_3. Feeding ECM more than 10% during the starter period retarded growth performance. Under unheated condition, birds can only be fed 10% ECM during day 29 to 35 for optimum performance. However, feeding 30% ECM was not detrimental for WG and FCR of heated chickens. Heat stress reduced percentage of carcass but increased leg meat yield. Diet had negligible effect on broiler carcass yield. Although feeding 20 and 30 % ECM elevated plasma level of T_3 in unheated birds, no effect of diet was observed on the hormone concentration in heated birds. Feeding FCM more than 10% during the starter period retarded growth performance. Both unheated and heated broilers can be fed 30% FCM without any detrimental effect on performance from 29 to 35 days of age. There was no significant effect of diet on carcass parameters or T_3 and T_4. Irrespective of diet, heat treatment reduced carcass weight and T_3. In conclusion, the present results suggest that extrusion and fermentation may improve the nutrient availability of CM. However, the optimum levels of ECM and FCM inclusion in the diets of broiler chickens varied according to age and environmental temperature. Cost effectiveness and practicality in terms of application under commercial setting are important factors to be considered in identifying the suitable method of treating CM.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

NILAI PEMAKANAN MIL KANOLA YANG DIFERMENTASI DAN DISEMPERIT SEBAGAI BAHAN MAKANAN AYAM DAGING DI BAWAH KEADAAN TIDAK PANAS DAN PANAS

Oleh

AHMED ABED WADI ALJUOBORI

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Pengerusi : Profesor Zulkifli B Idrus, PhD

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Kandungan asid amino di dalam kanola (CM) adalah setanding dengan kandungan protein di dalam soya. Walau bagaimanapun, ia mengandungi faktor anti-nutrisi dan kadar serat yang tinggi di mana boleh mengehadkan penggunaannya untuk makanan ayam pedaging. Oleh itu, adalah disarankan untuk mengkaji kaedah yang sesuai bagi meningkatkan penggunaan CM dalam pemakanan ayam, lebih-lebih lagi mengenai kandungan seratnya yang tinggi dan juga kegunaannya ketika beriklim panas. Kajian ini mengkaji kesan proses penyemperitan dan penapaian ke atas beberapa nilai nutrisi dan anti-nutrisi di dalam CM. Respon ayam pedaging terhadap beberapa jenis makanan iaitu; kanola (CM), kanola yang menjalani proses penyemperitan (ECM) dan kanola yang menjalani proses penapaian (FCM) dalam dua keadaan yang berbeza; iaitu ketika dikenakan haba dan tidak dikenakan haba telah dikaji dan dikenal pasti. Kajian pertama adalah untuk mengkaji kesan proses penyemperitan kepada nilai tenaga, protein dan asid amino di dalam CM. Keputusan menunjukkan bahawa ECM mempunyai nilai tenaga yang lebih tinggi berbanding CM (2595 vs 2243 kilo kalori/kg). Proses itu juga meningkatkan kadar penghadaman protein dan asid amino seperti Asp, Glu, Ser, Thr dan Trp di ileum. Kajian kedua pula untuk mengkaji potensi 'Lactobacillus' di dalam proses penapaian untuk mengurangkan kandungan glucosinate di dalam CM, seterusnya meningkatkan kadar penghadaman oleh ayam. Penghadaman nutrisi di ileum dikaji dengan menggunakan ayam berumur 42 hari dan keputusan menunjukkan kadar penghadaman protein dan asid-asid amino adalah lebih baik apabila ayam diberi makan FCM. Walau bagaimanapun, proses itu tidak memberi kesan kepada jumlah tenaga di dalam CM. Tiga eksperimen dijalankan untuk mengkaji kesan pemakanan CM, ECM dan FCM kepada prestasi ayam, nilai karkas, suhu badan ayam, tahap T_3 dan T_4 di dalam badan ayam. Ayam diberi makan CM yang diproses sejak berumur 1-35 hari; iaitu sebanyak 0%, 10%, 20% dan 30%. Kandungan protein dan tenaga di dalam makanan adalah sama. Dari hari ke-29 hingga ke-35, separuh bilangan ayam dikenakan haba sebanyak 36±1°C manakala separuh lagi bersuhu 23±1°C, berterusan sepanjang tempoh itu. Makanan yang menggunakan CM sebanyak 10% dan ke atas ketika fasa permulaan

telah merencatkan kadar penambahan berat badan ayam dan juga kadar penukaran makanan (FCR). CM yang ditambah sehingga 30% ketika fasa pengakhiran hanya boleh diberi makan kepada ayam yang dikenakan haba, tetapi tidak kepada kumpulan ayam yang sebaliknya. Nilai karkas, T_3 dan T_4 tidak terkesan oleh faktor pemakanan. Ayam yang dikenakan haba mempunyai nilai karkas yang lebih rendah. Kandungan FCM yang melebihi 10% semasa fasa permulaan merencatkan prestasi pertumbuhan ayam. Ayam yang tidak dikenakan haba hanya boleh diberi makan 10% ECM semasa hari ke-29 hingga ke-35 untuk prestasi optimum. Walau bagaimanapun, penggunaan 30% ECM kepada ayam yang tidak dikenakan haba tidak memberi kesan buruk kepada kadar pertumbuhan dan FCR. Tekanan haba mengurangkan hasil karkas tetapi meningkatkan hasil daging. Faktor makanan memberi kesan yang tidak penting (boleh diabaikan) terhadap hasil karkas ayam. Walaupun memberi makan ECM sebanyak 20% dan 30% meningkatkan tahap T_3 bagi ayam yang tidak dikenakan haba, faktor makanan tidak memberi kesan kepada jumlah hormon di dalam ayam yang dikenakan haba. Penggunaan FCM melebihi 10% semasa fasa permulaan merencatkan prestasi pertumbuhan. Kedua-dua kumpulan ayam; sama ada dikenakan haba atau tidak boleh diberi makan FCM sebanyak 30% semasa hari ke-29 hingga ke-35 tanpa memberi kesan yang buruk terhadap prestasi pertumbuhan. Jenis makanan tidak memberi kesan yang ketara terhadap nilai karkas, T_3 dan T_4. Tidak mengambil kira jenis makanan, tekanan haba mengurangkan nilai karkas dan T_3. Kesimpulannya, kajian ini menunjukkan bahawa proses penyemperitan dan penapaian telah meningkatkan jumlah nutrien di dalam CM. Walau bagaimanapun, kadar penggunaan ECM dan FCM yang optimum di dalam makanan ayam berubah mengikut umur dan suhu persekitaran. Keberkesanan kos dan sejauh mana kajian ini boleh dipraktikkan secara komersial adalah faktor penting yang patut dipertimbangkan dalam mengenal pasti kaedah yang sesuai untuk memproses CM.

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Ahmed Abed Wadi Aljuobori

2014

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AA	Amino Acids
AABA	L-α-Amino-n-Butyric Acid
ADF	Acid Detergent Fiber
AME	Apparent Metabolizable Energy
AMEn	Apparent Metabolizable Energy Corrected to Zero Nitrogen
ANOVA	Analysis of Variance
AOAC	Association Official Agricultural Chemists
BLAST	Basic local alignment search tool
BW	Body Weight
CF	Crude Fiber
CFU	Colony Forming Unit
CM	Canola meal
cm	Centimeter
CP	Crude Protein
CRD	Completely Randomized Design
DCP	Di Calcium Phosphate
DF	Dietary Fibre
DM	Dry Matter
DNA	Deoxyribonucleic acid
EAA	Endogenous Amino Acid
ECM	Extruded canola meal
EE	Ether Extract
FCM	Fermented canola meal
FCR	Feed Conversion Ratio
IU	International Unit
g	Gram
g	Unit for measuring centrifugation force
g/day	Gram per day
GE	Gross energy
h	Hour
H_2SO_4	Sulphuric acid
HCl	Hydrochloride acid
HPLC	High Performance Liquid Chromatography
Kcal/kg	Kilocalorie per Kilogram
LAB	Lactic acid bacteria
LVI	Limited volume insert
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimetre
mM	Mille mole
MRS	De Man, Rogosa and Sharpe
nmol/L	Nanomoles per liter

NCBI	National Center for Biotechnology Information
NDIN	Neutral detergent in-soluble nitrogen
ng	Nanogram
nm	Nanometer
NO	Nitric oxide
NRC	National Research Council
NSP	Non starch polysaccharide
ºC	Degree Celsius
PCR	Polymerase chain reaction
pH	Hydrogen ion concentration
pmol	Picomole
RH	Relative humidity
RIA	Radioimmunoassay
r-T_3	Reverse triiodothyronine
rpm	Revolutions Per Minute
rRNA	Ribosomal RNA
SAS	Statistical Analysis System
SEM	Standard Error of Mean
SSF	Solid State Fermentation
SmF	Submerged Fermentation
SM	Secondary metabolites
Ta	Ambient temperature
Tb	Deep Body Temperature
TEA	Triethlamine
TMA	Trimethylamine
T_3	3,3’,5-Triiodothyronine
T_4	Tetraiodothyronine
WG	Weight gain
µg	Microgram
µl	Microlitre
µmol/g	Micromoles per gram
v.	Versus

CHAPTER 1

INTRODUCTION

The largest single source of supplementary protein used for livestock and poultry feeding is soybean meal. In recent years, because of economical and agro-political reasons, it has become advantageous in most countries to replace soybean meal or part of it with locally produced protein rich vegetable feeds, usually referred to as soybean meal replacers. Among the popular alternative vegetable protein sources for poultry feed, canola meal is ranked second (Nowlin, 1991).

Canola is the name for a genetically altered form of rapeseed with less than two percent erucic acid and less than 30 micromoles of glucosinolate per gram of air-dried oil-free meal (Bell, 1993). The development of low erucic acid and low glucosinolate cultivars of canola has resulted in increased usage of canola meal in poultry rations (Robblee *et al.,* 1986). Canola is the most important oil and protein crop in Canada, due to its high yield and high nutritive quality of both oil and protein. The commercial canola meal usually is made from a blend of *Brassica napus* and *Brassica rapa* seed by a combination of expelling and solvent extraction, and it contains 34 to 38% crude protein and has a well balanced amino acid profile and high levels of sulfur- containing amino acids relative to soybean meal. It is rich in essential minerals and seems to be rich in choline, biotin, folic acid, niacin, riboflavin and thiamin (Bell, 1993). Although canola meal contains a high concentration of crude protein, it also contains antinutritional substances such as glucosinolate, fibre, phytate, sinapine and tannins. Even though canola has a low level of glucosinolate relative to the original rapeseed, the glucosinolate content is still considered to be at a level that influences its exclusive use in monogastric animals (Jia *et al.*, 2012). The suitability of raw canola meal as a source of protein in poultry feed is limited due to present of anti-nutritional factors such as glucosinolats and high fiber content which decrease the digestibility of canola meal. Glucosinolates content of canola meal depress the growth performance of broiler chicken by decreasing the feed intake, disrupting the thyroid hormones synthesis, liver and kidney degeneration, and inducing anemia (Tripathi and Mishra, 2007).

The high fiber level is also another major restriction to the use of canola meal in poultry diets. Dietary fiber accounts for approximately one-third of the meal and it consists of 13- 16% of non-cellulosic polysaccharides, 4-6% of cellulose, 5-8% of polyphenols and lignin and finally protein and minerals which related to the fiber content (Slominski and Campbell, 1990). Thus, the low metabolizable energy content and the less than optimal protein and amino acid digestibilities caused by the high level of dietary fiber. The non-starch polysaccharides which include cellulose, β-glucans, arabinoxylans, and pectins may increase the viscosity of digesta resulting in decreased nutrient digestibility (Annison, 1991), subsequently resulting in low growth performance, feed conversion ratio and digestibility of feed.

One of the possible approaches to diminish the antinutritional factor and enhance the bioavailability of nutrients in canola meal is by the extrusion process. Extrusion included pressure, heat treatment and shear force which has been reported to have beneficial effects on the nutritional value of feedstuffs (Allan and Booth, 2004; Burel *et al.*, 2000). Extrusion process increases the accessibility of proteins to enzymatic breakdown (Bhattacharya and Hanna, 1988). Another approach is to use microbial solid state fermentation to eliminate anti-nutritional factors and enhance bioavailability of nutrients. Marklinder *et al.* (1996b) demonstrated that, during lactic acid fermentation of cereal, composition of carbohydrate is modified due to microbial metabolism and intrinsic enzyme activation in the cereal. Skrede *et al.* (2003) showed that feeding fermented wheat and barley to chickens improved their growth and feed: gain ratio. As such, soybean meal (Gao *et al.*, 2013) , cottonseed meal (Zhang *et al.*, 2007), and agriculture by-products (Oduguwa *et al.*, 2008) are also being fermented to eliminate the anti-nutritional factors such as trypsin inhibitor, gossypol and to improve nutrient content and availability.

High ambient temperature reduces growth rate, feed consumption, and the survival of broiler chickens, which can decrease profitability (Dale and Fuller, 1980; Teeter and Belay, 1996; Donkoh, 1989). Moreover, broilers raised under heat stress have increased abdominal fat (McNaughton and Reece, 1984). The time required to reach the desired weight is also adversely affected (Ahmad and Sarwar, 2005) during periods of high temperature.

Broiler production in Asia, Africa and South America will continue to maintain its strong growth rate. However, production performances in these regions remain relatively inferior compared to those achieved in temperate countries. Although, several factors are clearly involved, the combination of high prices of soybean meal and high temperature remains the major constraint to enhance the production efficiencies in warm regions. The high prices of soybean meal reduce the usage in poultry diets. Canola meal, as an alternative, is available at lower prices and thus can be used to reduce the cost of final diet (Aftab, 2009). It is recommended to limit the use of fibrous ingredients in poultry diets under heat stress conditions (Leeson and Summers, 1997). High dietary fiber diets may also reduce the availability of essential amino acids in heat-stressed chickens and thus their performance (Koelkebeck *et al.*, 1998). Consequently, the maximum safe level of inclusion of canola meal in broiler diets under heat stress is a governing factor.

Thus, it is a novel approach to determine the nutritive value of extruded (ECM) and fermented (FCM) canola meals and the safety of their inclusion in diets under normal and high ambient temperature. Therefore the present experiments were conducted to evaluate and optimize the potential of extrusion and fermentation treatment on efficacy of feeding canola to broiler chickens.

General objectives

(i) To determine the effect of physical treatment by extrusion on the chemical composition, AME and nutrient and amino acid digestibility of CM and ECM in broilers.
(ii) To evaluate the biological treatment of CM by solid state fermentation with lactic acid bacteria in reducing glucosinolate present in canola meal and enhancing nutrients digestibility for broiler chickens.

Specific objectives

i. To evaluate the effect and interactions of different levels of untreated CM on growth performance, carcass yield, and blood concentration of triiodothyronine and tetraiodothyronine of male broilers during the period of chronic heat stress.

ii. To evaluate the effect and interactions of different levels of ECM on growth performance, carcass yield, and blood concentration of triiodothyronine and tetraiodothyronine of male broilers during the period of chronic heat stress.

iii. To determine the effect of solid state fermentation on nutrient content and amino acid digestibility of CM in broilers.

iv. To evaluate the effect and interactions of different levels of FCM on growth performance, carcass yield, and blood concentration of triiodothyronine and tetraiodothyronine of male broilers during the period of chronic heat stress.

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